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FEATURE ARTICLE

Amphibian declines and possible etiologies: the case for Sri Lanka

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A group of New Country middle school children and their teacher conducting a study of wetland ecology started collecting leopard frogs from a small pond in Minnesota, USA. Of the frogs they caught that day, half were severely deformed. Most had deformities associated with the hind limbs. Some had five or more hind limbs, others had twisted legs, no hind limbs at all or had mere nubbins where legs should be. In some, one or two legs sprouted from the stomach. Few were without an eye. This story, which appeared in a local newspaper in 1995, received national media attention in the US. Reporters and scientists country wide arrived in Minnesota to see these deformed frogs. This story raised two main questions: whether these deformities were an isolated occurrence or a widespread phenomenon, and what caused the deformities. To answer the first question, researchers mainly in the US and also in other countries started investigating their local amphibian populations and found that deformed frogs are not confined to Minnesota. It is a widespread phenomenon. Since 1995, deformities have been reported in more than 60 species of amphibians including salamanders and toads in 46 states and surprising numbers of deformed amphibians have been found in Asia, Europe and Australia[1].

Finding an answer to the second question, what causes the deformities, was more difficult. Field and laboratory studies to identify what deformed the frogs suggest that many complex causes are involved, the prime suspects being parasitic infections, UV radiation and biocides[1]. While the amphibian deformities became a hot topic only after mid 1990s, the amphibian declines had been known for more than three decades. The global loss of amphibian populations was first recognized in 1989[2]. By 1993, more than 500 populations of amphibians were reported to be in decline or were listed endangered[3]. Recent studies show that global amphibian losses are the result of interactions between a number of highly context-dependent causal factors. Main causes of population declines seem to stem in part from human activities, which have resulted in habitat loss and alterations, global warming, chemical contaminants, and acid rains together with invasive species and retinoid mimics[4,5,6]. Some findings about the decline of wild frog populations are enigmatic. In some cases, frog populations or entire species in remote pristine areas or virgin forests suddenly vanished in a few months. The Australian brooding frog (*Rheobatrachus silus*), has not been observed since 1981 and is presumed to be extinct. This species had inhabited a relatively undisturbed tropical rainforest[7,8]. Baffled by the vulnerability of frogs in protected areas, scientists have investigated the effects of a large number of environmental factors, ranging from increased ultraviolet (UV) light to natural enemies like fungal, viral and parasitic infections. Epidemiologists say that Chytridiomyosis[9] caused by a virulent fungus may be a key factor in the sudden, mysterious decline of frogs around the globe particularly those from wilderness areas. Virulent fungi have been shown to kill spawn and tadpoles as well as adult amphibians. The mechanism by which this chytrid fungus kills frogs is unknown. The fungus may impair cutaneous respiration and osmoregulation or may cause death due to absorption of toxic products released by the fungus[9].

An early suspect of a possible cause of amphibian deformities was the excess exposure to UV radiation resulting from the thinning ozone layer. UV radiation had already been identified as a cause of amphibian declines. When tadpoles of the western toad, *Bufo boreas* were exposed to enhanced UV-B radiation they developed deformities and had lower survival rates[10]. The northern leopard frog *Rana pipiens* also developed hind limb deformities when exposed to UV radiation[11]. These UV radiation induced hind limb malformations in *Rana pipiens* were dose dependent[12]. However, UV radiation does not explain all the types of limb deformities seen in nature.

A connection between trematode infection and amphibian deformities was first hypothesized13 during an investigation of an outbreak of deformities among populations of Pacific tree frogs (*Hyla regilla*) and long-toed salamanders (*Ambystoma macrodactylum*). Numerous metacercariae were found to concentrate around the deformed limbs. Experimental implantation of metacercariae-sized resin beads into the developing limb buds of *Xenopus laevis* induced some deformities supporting their hypothesis13. Subsequent field studies14 identified a positive correlation between the deformities and the trematode *Ribeiroria ondatrae* infections in *H. regilla* from northern California, USA. In later studies the western toad, *Bufo boreas* was exposed to the same parasite under laboratory conditions. The result was severe limb deformities identical to those observed at field sites15. A survey of five *Rana* species, two newts, one salamander and one toad species in western US has recorded deformities in all these species16.

Digenetic trematodes have an indirect life cycle involving three hosts. The adult parasite lives in birds, the definitive hosts, laying their eggs inside the bird. The eggs pass out with faeces into a pond or wetland. The miracidia that hatch out in water, seek out and enter a snail, the first intermediate host. The miracidia larvae stay inside the snail for a period of few weeks, multiply by passing through many polyembryonic stages and come out as free swimming cercariae. Cercariae actively invade a second intermediate host, a tadpole inside which they encyst and become metacercariae. Most frequently they encyst in the developing limb buds and thereby interfere in limb development. The parasite causes limb deformities in the frog, often resulting in extra or missing limbs. Such deformed frogs with the encysted cercariae become prime prey for wading birds, such as herons or egrets, the definitive hosts of the parasite, and thereby complete its life cycle.

Later, studies have pointed to parasite-induced limb deformities being influenced by human involvement[17]. A connection has been established between eutrophication caused by increased nutrients, the snail and the parasite[17]. It was found that snails were more abundant in ponds receiving runoff rich in nitrogen and phosphorus from agricultural fertilizers and animal waste.The study concluded that heavy fertilizer use had created an ideal environment for the parasite to expand its range[17].

Other causal agents deforming amphibians are chemical contaminants mainly pesticides, herbicides, fungicides, insecticides, and fertilizers[18]. Toxic substances can severely affect amphibians in a variety of ways. They can kill amphibians directly, affect their behaviour, reduce their growth rate and induce deformities[3]. Even though chemical contaminants contribute largely to the amphibian population declines, they alone do not explain all the deformities observed in natural amphibian populations.

Trematode infection has been identified as the principal cause of amphibian limb deformities while the effects of UV radiation and biocides are also making their impact. Several studies have examined the synergistic interactions between these three factors illustrating the importance of understanding how complex interactions affect the individual animals and perhaps ultimately, whole populations. Studies on the synergistic effect of trematode infections and pesticide exposure on malformations in wood frog *Rana sylvatica,* showed that trematode infected frogs exposed to pesticides experienced much higher levels of deformities[19]. UV radiation could also transform pesticides into teratogens, which are chemicals that can interfere with development and cause birth defects.

There is speculation as to whether parasite-induced limb deformities contribute to amphibian population declines. Population decline and deformities in frogs are two distinct problems[20]. Theoretically, however, overlap is possible in some instances. For example, the increase in parasite density not only increased the frequency of limb deformities but was also associated with decreased tadpole survivorship[14,16]. Furthermore, deformed frogs, in particular those with hind limb abnormalities may become easy prey[21] undoubtedly affecting their survival. Even though data from several studies suggest that deformities increase mortality rates, there is no information available to ascertain whether this affects population levels. In many cases an individual factor alone may not affect the amphibian population. However, given the complexity of dynamic ecological systems, simultaneous exposure to numerous agents and interactions between these could affect the amphibians at the population level.

Although deformed frogs received early attention in various countries mainly in the US and Canada, no significant attention has been paid to the deformities among amphibian populations in Sri Lanka. Sri Lanka is an amphibian hotspot in the world with very high species diversity per unit area, with a remarkably high amphibian endemicity. There are three genera and 87 species endemic to the island[22]. Most of the amphibian fauna are confined to the rain forests of the island's wet zone, which receives an annual rainfall in excess of 2000 mm. Many species in Sri Lanka remain undiscovered because their habitats remain largely unexplored. Up to date, 103 species of amphibians have been recorded in Sri Lanka[22] belonging to the four families Ichthyophidae, Microhylydae, Bufonidae and Ranidae.

Most of the amphibians start their lives as aquatic eggs and larvae and later switch to a terrestrial adulthood, thus, they experience two potentially polluted habitats.

In Sri Lanka, frogs lay eggs in many different habitats. These include irrigation reservoirs in the dry zone, flood lakes in the dry zone (formed as temporary wetlands due to flooding during rainy seasons which disappear at the end of the rainy season), pools associated with rivers where water is retained for a prolonged period, wet lands (villus) in the coastal dry zone such as Vilpaththu National Park, paddy fields (receiving water from lakes in the dry zone or rain water from wet zone, reservoirs, cascades) and also less favourable habitats such as estuaries and lagoons. The majority of rhacophorid tree frogs (Family Ranidae) in Sri Lanka show direct development, which probably restricts formation of deformities observed in species with aquatic eggs and larvae.

There are large numbers of small low-land wet zone forest reserves which are separated from each other by large tracts of cultivated lands. These reserves have high amphibian diversity and provide interesting areas to study the dynamics of amphibian populations[23]. Species such as *Bufo melanostictus, Polypedates cruciger* and *P. maculates* are mostly associated with human-modified habitats and are known to have expanded natural ranges and established a higher relative dominance following habitat disturbances[23]. These species are ideal candidates for laboratory studies of the development of deformities as they have shown to be less sensitive to the perturbations in the environment and their eggs are easily accessed.

More than 80% of the island's biodiversity rich wet zone forests have been lost during the last 150 years [24]. This includes part of anuran diversity as well where widespread extinctions had taken place in the Sri Lankan amphibian fauna[23]. Nineteen species are already extinct while 11 species are critically endangered[25]. Undoubtedly, many factors are contributing to the decline of the local populations, and it is important that we identify the key contributors to our species decline in Sri Lanka.

Here we report the first field observations of deformed amphibians in Sri Lanka from two protected forests. Specimens of an endemic monotype genus *Lankanectes corrugatus* and the common *Rana temporalis* (Subfamily Raninae; Figure 1A and 2A) were found in a protected area in the Dothalugala Man and Biosphere Reserve of the Knuckles forest range which is a sub montane forest 1200 m above sea level. The stream where these specimen were found (3A) also had several species of fresh water snails. The deformed specimen of *R. temporalis* was a mature female with a very short right femur (ectromelia). Since there is a bulge at the hip it can be assumed that a portion of the femur is present (Figure, 1A). The deformed specimen of *Lankanectes corrugatus* showed both short toes (brachydactyly) and a short tibia and fibula (hemimelia; Figure 2B, 2C). In the brachydactylous condition, the normal number of metatarsals is present but the number of phalanges is reduced. In the hemimelia condition, the tibia and fibula are short but distal limb and the foot are present. Another specimen of *R. temporalis* was found from an abandoned man made water pool in the border of the Gannoruwa Forest Reserve (Figure 3B), which showed ectromelia of the tibio-fibula (Figure 1B). The stifle (knee) is present; therefore a portion of the tibio-fibula must be present. The Gannoruwa Forest Reserve is a low land semi-evergreen forest at 680 m above mean sea level.

The widespread occurrence of these deformities in natural populations of amphibians requires further investigations. Another important area of research which should be pursued is how the trematode species found in Sri Lanka affect the development of abnormalities in local amphibian species. Rajakaruna and co-workers (manuscript in preparation) have shown that under laboratory conditions, exposure to monostome type cercariae, leads to severe deformities, in *P. cruciger*, the common hourglass tree frog, in a dose-dependent manner (Figure 4; manuscript in preparation). Further experiments are underway exposing another amphibian species, the common toad *Bufo melanostictus (Ranidae)* to the same infection.

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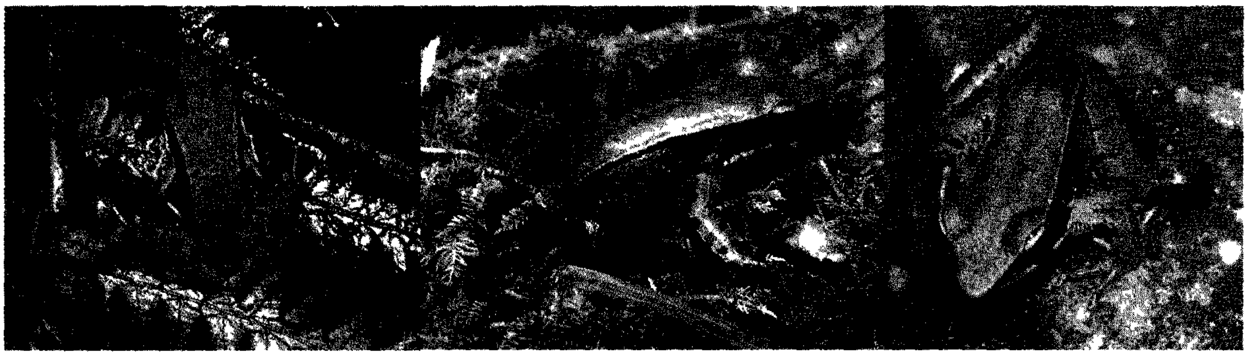


A B C

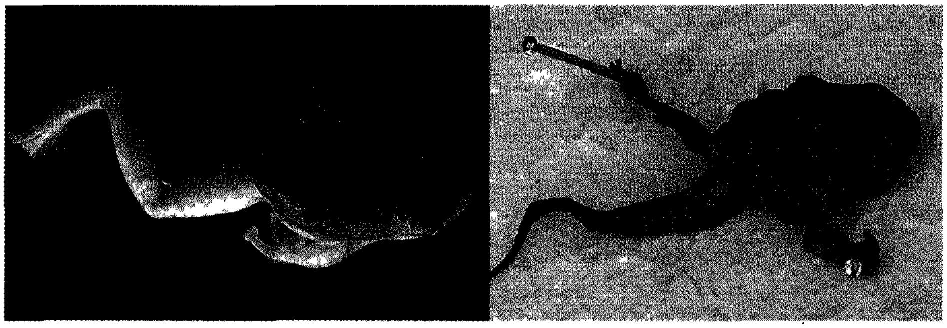
Figure 1: *Rana temporalis* from the Knuckles wilderness (A) a frog with no deformities (B) frog with ectromelia of femur Note that the right femur is very short and difficult to discern, but since there is a bulge at the hip it can be assumed that a portion of the femur is present (C) *R temporalis* from the Gannoruwa Forest Reserve with ectromelia of the tibio-fibula The stifle (knee) is present, therefore a portion of the tibio-fibula must be present

A B C

Figure 2: Deformed *Lankanectes corrugatus* from the Knuckles wilderness (A) a toad with no deformities, (B) deformed frog with short toes (brachydactyly) The normal number of metatarsals is present but the number of phalanges is reduced (C) deformed toad with short tibia and fibula (hemimelia)

A B

Figure 3: Natural water bodies where the deformed frogs were found (A) a stream in the Knuckles wilderness and (B) an abandoned man-made pond in the Gannoruwa Forest Reserve



A B

Figure 4: Severe deformities observed in common hourglass tree frog, *Polypedates cruciger* after exposure to the monostome type cercariae under laboratory conditions (Rajakaruna *et al.*, manuscript in preparation). (A) Left hind limb absent (amelia) and lack of pigmentation in the limbs. (B) Severely malformed specimen showing absence of digits (ectrodactyly), absence of limb (apody), partially developed limbs (hemimely), absence of right fore limb (amelia). Classification of malformations were done according to the United States Geological Survey (USGS) Field Guide to Malformations of Frogs and Toads[26].

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